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Research Article

Seed apex curvature in key Spanish grapevine cultivars

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Abstract

New methods of image analysis may contribute to the morphological description of seed shape and cultivar classification in Ampelography. We recently proposed a method based on the comparison of seed images with geometric figures, defined by mathematical equations for the identification and classification of grapevine cultivars. Representative cultivars of *Vitis vinifera*, conserved in the Spanish collection of IMIDRA, were classified in ten groups according to the similarity of their seeds with geometric models. Complementary to this method, we propose here the analysis of curvature in the seed apex and the quantification of curvature values in the seed images. Curvature is a parameter that indicates the variation of the slope values along the points of a continuous curve. A set of points along the surface of the seed images are marked and used to obtain the Bézier Curves corresponding to seed profiles. The curvature values along the curves are calculated and represented. According to the variation of their curvature and distribution of maximum curvature values, the cultivars are classified in three groups. The groups formed on the basis of curvature analysis are related to the classification based on geometrical figures. Curvature in the apex describes an important morphological aspect at the basis of the classification by shape.

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Keywords

Ampelography, curvature, geometry, grapevine, morphology, beak, shape, taxonomy

Introduction

The description, classification and phylogenetic relationships of grape cultivars belong to Ampelography, a discipline traditionally based on the analysis of morphological characters (Viala and Vermorel 1901). The shape and colour of the vine leaves, grape clusters and berries provided the basis for the identification and classification of the varieties of grapevines. In the last decades, the development of molecular techniques, based on the analysis of DNA polymorphisms, has displaced morphological techniques in Ampelography (Arroyo-García et al. 2006, Arroyo-García et al. 2002, Barth et al. 2009, Lopes et al. 1999, Sefc et al. 2009, Lefort and Roubelakis-Angelakis 2001, Maletic et al. 1999), but nevertheless, the recent developments in image analysis can restore morphology to an important source of information for Ampelography (Christodoulou et al. 2020, Terral et al. 2009, Ucchesu et al. 2016, Martín-Gómez et al. 2020b, Pagnoux et al. 2014).

In a recent study with 38 cultivars of the IMIDRA collection, ten groups were proposed according to the similarity of their seeds with geometric figures (Cervantes et al. 2021). The groups were nominated, based on the names of representative cultivars: Listán Prieto, Silvestris, Albillo Real, Hebén, Moscatel, Tortozón, Teta de Vaca, Doña Blanca, Airén and de Cuerno (Cervantes et al. 2021). The groups are ordered in this list by decreasing Stummer Index (width/length) (Stummer 1911) and each of them contains those cultivars whose seeds gave high percentage of similarity to a corresponding geometric model. Similarity between the seeds and the models was based on the *J* Index, a parameter that quantifies the percentage of area shared between seed images and geometric figures (Cervantes et al. 2016, Cervantes and Gómez 2019). The *J* Index was first described in the model plant *Arabidopsis thaliana*, whose seeds resemble a cardioid elongated by a factor of Phi (The Golden Ratio) (Cervantes et al. 2010, Martín et al. 2014) and, later on, in the model legumes *Lotus japonicus* and *Medicago truncatula*, whose seeds resemble the cardioid and modified cardioids, respectively (Cervantes et al. 2012). The cardioid was the geometric figure used for seed shape quantification in *Capparis spinosa* (Saadaoui et al. 2013) and quantification by the *J* Index was reported also in the seeds of a variety of species using different geometric models (Saadaoui et al. 2017, Saadaoui et al. 2015, Martín Gómez et al. 2019, Cervantes and Martín Gómez 2018, Martín-Gómez et al. 2019, Gutiérrez del Pozo et al. 2020, Martín-Gómez et al. 2020a, Gómez et al. 2016).

We present here a new method to describe and quantify seed shape in the Vitaceae, complementary to the similarity of the seeds with geometric figures. The method is based in the obtention of Bézier Curves (Bézier 1968) corresponding to the seed silhouettes and subsequent curvature analysis of the seed apex at the beak tip (beak, pedicel and peduncle are terms used indistinctly and both refer to the apical part of the seed) (Weisstein 2007, Weisstein 2011, Noriega et al. 2009, Noriega et al. 2008, Tocino and Cervantes 2008, Cervantes and Tocino 2005).

The curvature of a plane curve is a parameter which measures how the direction (slope) of the curve changes along the successive points (Weisstein 2007). Images of the surface of root and seed apex can be converted to curves and the variation of their slopes (curvature values) calculated (Weisstein 2011, Tocino and Cervantes 2008, Noriega et al. 2008, Noriega et al. 2009, Cervantes and Tocino 2005). By this method, we could demonstrate that curvature values in the root apex were lower in ethylene-insensitive mutants of *Arabidopsis*, as well as in roots treated with peroxide (Cervantes and Tocino 2005, Noriega et al. 2008, Noriega et al. 2009, Tocino and Cervantes 2008). Curvature analysis in the seed apex reveals different seed types according to their degree of variation of curvature. Seeds of type 1) have a defined maximum curvature point, seeds of type 2) have two equivalent points of maximum curvature values and seeds of type 3) are of an intermediate shape, consisting in a point with maximum curvature value and a shoulder. These three types are present in varying proportions in the different cultivars and some of them are predominant in each of the groups defined by comparison with models (Cervantes et al. 2021). Curvature analysis, in combination with their similarity to geometric models, are useful tools in the classification of cultivars, based on the morphological properties of their seeds, thus contributing to their identification.

Materials and Methods

Plant material

The thirty eight cultivars used in this study are presented in Tables 2, 1. Table 1 contains the list of cultivars with the ampelographic description of their seeds, based on the OIV "Descriptor List for Grape varieties and *Vitis* species" (OIV codes 241-244). Table 2 shows the cultivars grouped according to their similarity with morphological models. A more detailed description is given in Cervantes et al. (Cervantes et al. 2021).

Seed images

Photographs were taken with a camera Nikon D80 of 10.2 megapixels. The images containing 30 seeds per cultivar were described in Cervantes et al. (2021) and are stored in Zenodo: https://zenodo.org/record/4433813#.X_7hiOhKh9A

Table 1.

List of cultivars used in this work with the ampelographic description of their seeds, based on the OIV "Descriptor List for Grape varieties and *Vitis* species" (OIV codes 241-244).

Cultivar	OIV-241 Formation of seeds (1-absent; 2-rudimentary; 3- well formed)	OIV-242 Length of seeds (1-very short; 3- short; 5-intermediate; 7-long; 9-very long)	OIV-243 Weight of seeds (1-very low; 3- low; 5-intermediate; 7-high; 9-very high)	OIV-244 Transversal ridges on dorsal side of seeds (1- absent; 2-present)
Airén	3	5	3	1
Alarije	3	3	3	1

Cultivar	OIV-241 Formation of seeds (1-absent; 2-rudimentary; 3- well formed)	OIV-242 Length of seeds (1-very short; 3- short; 5-intermediate; 7-long; 9-very long)	OIV-243 Weight of seeds (1-very low; 3- low; 5-intermediate; 7-high; 9-very high)	OIV-244 Transversal ridges on dorsal side of seeds (1- absent; 2-present)
Albillo Real	3	3	5	1
Beba	3	3	5	1
Bobal	3	5	3	1
Bruñal	3	3	3	1
Caíño Tinto	3	5	3	1
Castellana Blanca	3	5	5	1
Cayetana Blanca	3	5	3	1
De Cuerno	3	9	3	1
Dominga	3	5	5	1
Doña Blanca	3	5	3	1
Garnacha Tinta	3	3	2	1
Gewürztraminer	3	3	3	1
Graciano	3	3	3	1
Hebén	3	5	5	1
Imperial	3	3	5	1
Juan García	3	5	5	1
Listán Prieto	3	3	5	1
Macabeo	3	3	3	1
Malvasía Aromática	3	5	3	1
Mazuela	3	3	3	1
Mollar Cano	3	5	5	1
Monastrell	3	5	5	1
Moscatel de Alejandría	3	3	5	1
Moscatel de Grano Menudo	3	3	3	1
Palomino Fino	3	3	3	1
Pedro Ximénez	3	5	3	1
Prieto Picudo	3	3	3	1
Sylvestris CA 13,4	3	3	3	1
Sylvestris CA 13,6	3	3	3	1
Sylvestris SE 2,1	3	3	2	1

Cultivar	OIV-241 Formation of seeds (1-absent; 2-rudimentary; 3- well formed)	OIV-242 Length of seeds (1-very short; 3- short; 5-intermediate; 7-long; 9-very long)	OIV-243 Weight of seeds (1-very low; 3- low; 5-intermediate; 7-high; 9-very high)	OIV-244 Transversal ridges on dorsal side of seeds (1- absent; 2-present)
Tempranillo	3	3	3	1
Teta de Vaca	3	5	3	1
Tortozón	3	5	3	1
Tortozona Tinta	3	3	7	1
Verdejo	3	5	3	1
Zalema	3	3	3	1

Table 2.

Groups of cultivars according to their similarity with morphological models. The groups are ordered by increasing aspect ratio (length/width). Underlined: cultivars used in the whole seed curvature analysis. Between parenthesis: Number of seeds used in the peduncle curvature analysis.

Model	**Cultivars**
Listán Prieto	Listán Prieto (15), Tortozona Tinta (15)
Sylvestris	CA 13.4 (29), CA 13.6 (30), SE 2.1 (30)
Albillo Real	Alarije (29), Albillo Real (30), Cayetana Blanca (10), Graciano (15), Juan García (10), Tempranillo (10)
Hebén	Hebén (30), Macabeo (30), Zalema (30)
Moscatel	Beba (12), Bruñal (15), Caíño Tinto (10), Castellana Blanca (30), Garnacha Tínta (30), Gewürztraminer (15), Malvasía Aromática (15), Mollar Cano (15), Moscatel de Alejandría (15), Moscatel de Grano Menudo (14), Palomino Fino (20), Prieto Picudo (15)
Tortozón	Imperial (30), Tortozón (30)
Teta de Vaca	Dominga (30), Teta de Vaca (30), Verdejo (30)
Doña Blanca	Doña Blanca (5), Monastrell (13), Pedro Ximénez (20)
Airén	Airén (30), Bobal (20), Mazuela (39)
De Cuerno	De Cuerno (5)

Curvature analysis

Seed images were inserted in Mathematica files (.nb). The analysis of curvature comprised two parts. First, it was applied to whole seed images. Points were taken along the seed silhouettes and the corresponding Bézier Curves were obtained. The maximum curvature values, corresponding to the beak tip, were calculated according to Tocino and Cervantes (Tocino and Cervantes 2008) (Fig. 2). The second task involves the analysis of curvature in the apex of the pedicel. It was based on the same protocol. A series of points defining the

contour of the root apex at the pedicel were marked. The number of points oscillated between 18 and 30, with lower numbers in cultivars with smaller beak. Bézier Curves were approximated for each set of points corresponding to the apex and graphic representations, corresponding to the respective curvature values of the Bézier Curves, were obtained Tocino and Cervantes 2008) (Fig. 1).

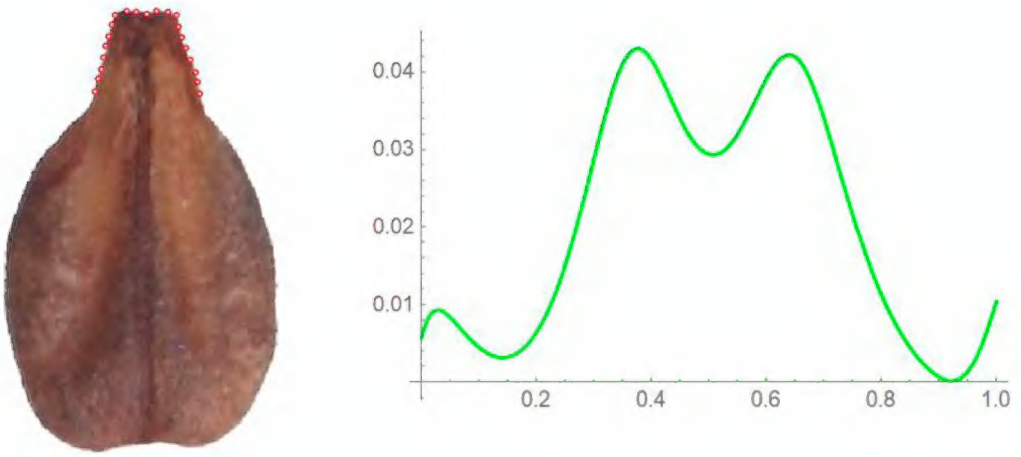

Figure 1. doi

Conversion of a seed peduncle image to the corresponding Bézier Curve and calculation of the curvature values. Seed images were inserted in Mathematica notebook (.nb). A set of points was marked on the profile of their beaks (left). The coordinates of the points were obtained and run in a programme (Tocino and Cervantes 2008) to obtain the corresponding Bézier Curves and the graphs representing the corresponding curvature values (right).

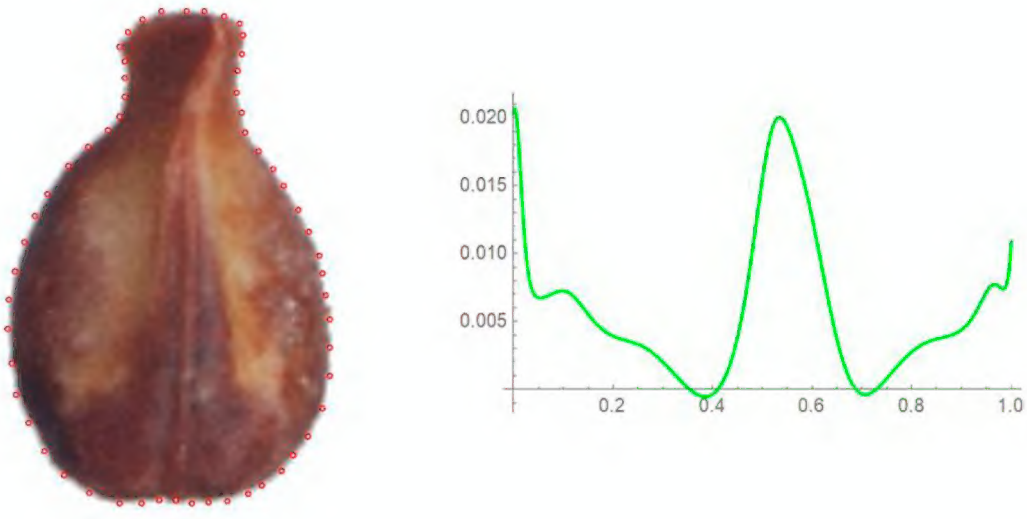

Figure 2. doi

A seed image was converted to the corresponding Bézier Curve and the curvature values calculated. Seed images were inserted in Mathematica notebook (.nb) and a set of points was marked defining the profile of the whole seed (left). The coordinates of the points were obtained (b) and run in a programme (Tocino and Cervantes 2008) to obtain the corresponding Bézier Curves, from which graphs representing the corresponding curvature values were obtained (right).

Difficulties encountered with the application of this protocol derive from structural aspects of *Vitis* seeds. First, some seeds have their beak curved towards one or the other side and this results in complex curves with variable results. This occurs particularly in some of the cultivars, for example, Juan García. In addition, the beak is a tissue rich in mucilage and often debris from the epidermis or cuticle fragments remains attached making difficult the clear delineation of the profile of the seed at the top. Thus, the number of seeds analysed per cultivar was variable (Table 2). In some cultivars whose seeds are uniform, symmetric and without cell debris or mucilage, a low number of seeds was found to be sufficient for the analysis (i.e. Listán Prieto, Tortozona tinta, Tempranillo and Caíño tinto).

Results

This section is divided in five parts: Curvature analysis in whole seeds; seed size, shape and peduncle structure in *Vitis vinifera* subsp. *sylvestris*; Variation in the peduncle in cultivars Dominga, Teta de vaca, Verdejo, Bobal, Hebén and Moscatel de grano menudo; Cultivars with an acute beak: de Cuerno, Imperial, Macabeo, Mazuela, Tortozón, Zalema and Cultivars with a plane or obtuse beak: Airén, Alarije, Albillo Real, Beba, Bruñal, Caíño Tinto, Castellana Blanca, Cayetana Blanca, Doña Blanca, Garnacha Tinta, Gewürztraminer, Graciano, Juan García, Listán Prieto, Malvasía Aromática, Mollar Cano, Monastrell, Moscatel de Alejandría, Palomino Fino, Pedro Ximénez, Prieto Picudo, Tortozona Tinta, Tempranillo.

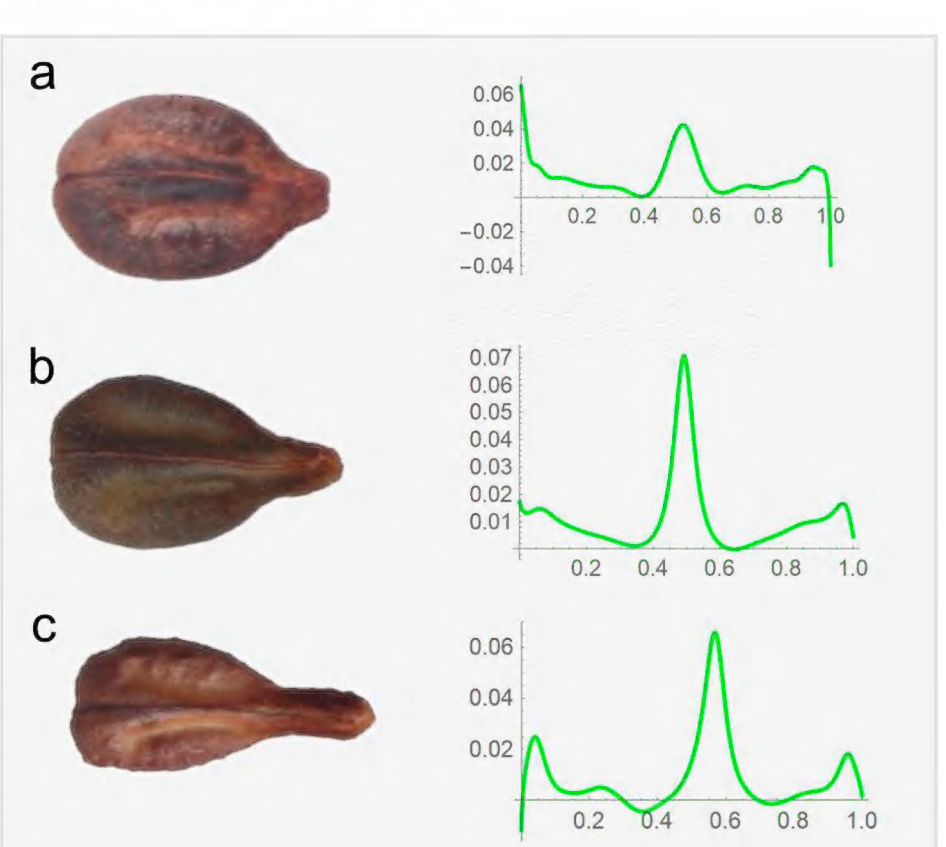

Figure 3. doi

Seed images and graphic representation of curvature values in whole seeds of: **a.** CA 13.4; **b.** Tortozón; **c.** de Cuerno. Seed images were inserted in Mathematica, a set of points was marked on their profile, the coordinates of the points were obtained and run in a programme (Tocino and Cervantes 2008) to obtain the Bézier Curves and graphs representing the corresponding curvatures.

Curvature analysis in whole seeds

Fig. 3 and Fig. 4 show seed images and the results of curvature analysis in whole seeds of some cultivars representative of the morphological groups. The method allows to distinguish between seeds of different groups based on maximum curvature values. Nevertheless, the method becomes more precise when the analysis is concentrated on the peduncle.

Figure 4. doi

Seed images and curvature values in whole seed images of: **a.** Tempranillo; **b.** Tortozona Tinta; **c.** Bruñal; **d.** Doña Blanca. Seed images were inserted in Mathematica, a set of points was marked on their profile, the coordinates of the points were obtained and run in a programme (Tocino and Cervantes 2008) to obtain the Bézier Curves and graphs representing the corresponding curvatures.

Seed size, shape and peduncle structure in *Vitis vinifera* subsp. *sylvestris*

The seeds of CA 13.4, CA 13.6 and SE 2.1 were colected from wild populations close to the Nateruela stream in Cádiz and river Huéznar in Seville respectively. They are smaller and more regularly-shaped (rounded or ovoid) than the seeds of the majority of cultivars used in Viticulture. Seed shape in these three varieties adapts well to a model oval or pyriform, with an elongated part corresponding to the beak that gives them the apparence of a fig Cervantes et al. 2021. The shape of the pedicel in seed images of these cultivars changes with the point of view. In a frontal view, it is plane or slightly bifurcated at the top and, in consequence, the corresponding curve has two points of maximum curvature Fig. 5(a). But if the point of view is displaced towards an upper or lower position, or under an assymmetric presentation of the seed with the rib in a lateral position, the upper part describes a continuous curve with a unique point of maximum curvature Fig. 5(b). This difference observed in shape with an slight change in perspective applies to the seeds of CA 13.4, CA 13.6 and SE 2.1. In well oriented, symmetric seeds, the Bézier

representations have two curvature peaks, either with two maximum curvature values of equivalent magnitude Fig. 5(a), or slightly different Fig. 5(c). Also, a dominant peak with the maximum curvature value and a shoulder may be observed Fig. 5(d).

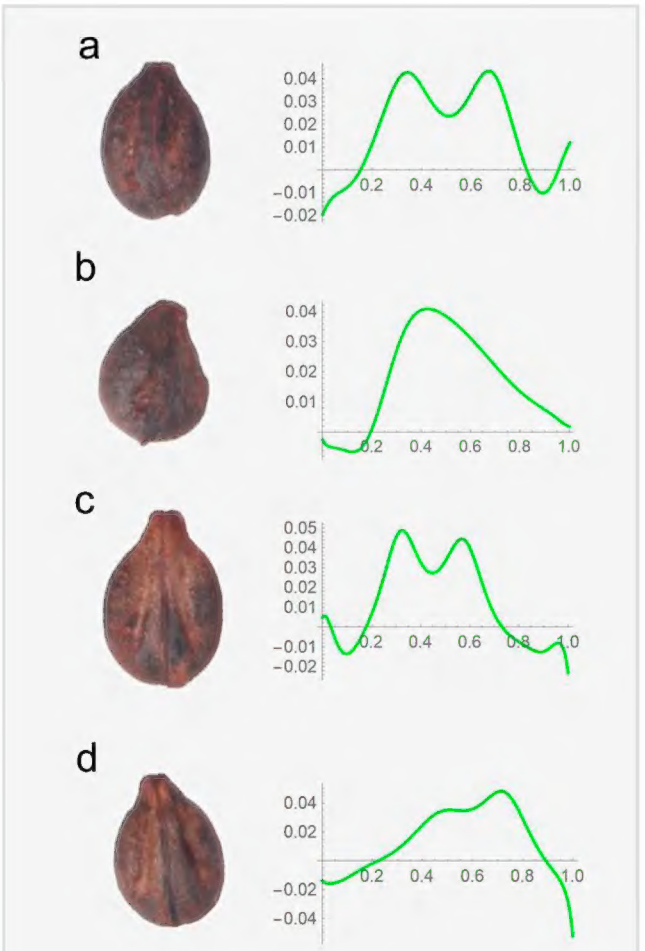

Figure 5. doi

Seed images and curvature values in the seed peduncle of wild-type cultivars (Sylvestris group): **a.** CA 13.4 seed in a frontal view, two maximum points of curvature are observed; **b.** A slight displacement of the point of view in seeds of the same cultivar results in a unique maximum curvature point; **c.** Images of a peduncle with two points of slightly different curvature values; **d.** A point with maximum curvature and a shoulder.

Variation in the peduncle in cultivars Dominga, Teta de vaca, Verdejo, Bobal, Hebén and Moscatel de grano menudo

Cultivars Dominga, Teta de vaca and Verdejo are in the same group in the classification by similarity to geometric models (Cervantes et al. 2021). Teta de vaca is an ancient variety already mentioned in latin texts and it was cultivated in private gardens in the Renaissance and the Baroque (Ponce Cárdenas and Rivas Albaladejo 2018). The beak in these varieties is broad at the basis, decreasing constantly in width towards the apex, giving to the seed a triangular aspect, also observed in particular seeds of other varieties (for example Airén, Bobal, Cayetana Blanca, Doña Blanca, Hebén and Zalema). Variation in the morphology of the beak is due to assymmetry that sometimes results from the accumulation of fragments of the cuticle in either one or the other side. The apex may be acute, resulting in a curve with a single maximum of curvature or plane, with two peaks of curvature or a peak and a shoulder (Fig. 6).

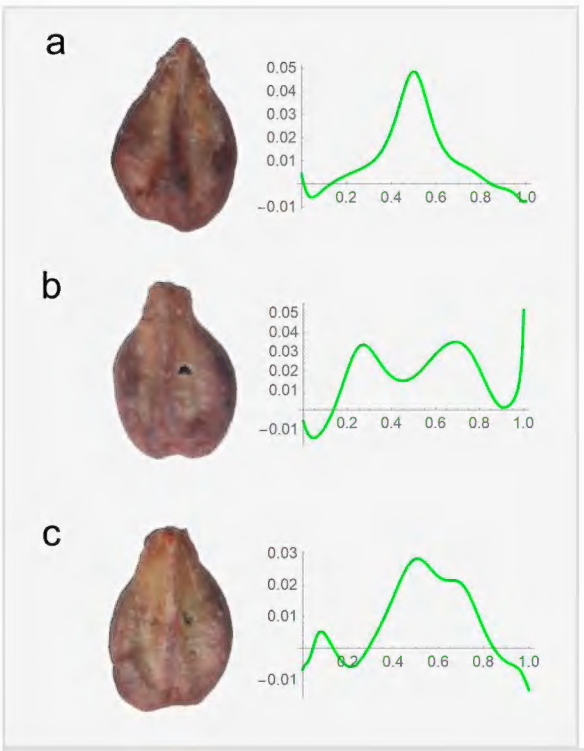

Figure 6. doi

Seed images and curvature values in the seed peduncle of cv. Teta de Vaca. **a.** Seed in a frontal view with a unique point of maximum of curvature; **b.** Seed in a frontal view showing two equivalent points of maximum curvature; **c.** Seed in a frontal view showing a point of maximum curvature and a shoulder.

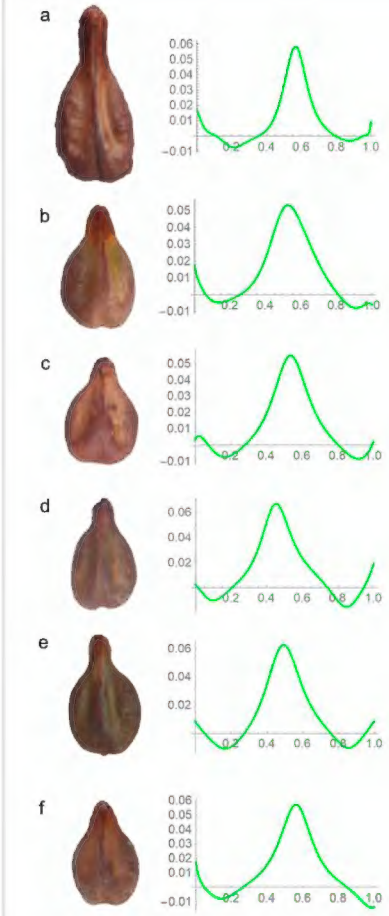

Figure 7. doi

Seed images and curvature values in the seed peduncle of (cultivars from top to bottom): **a.** De Cuerno; **b.** Imperial; **c.** Macabeo; **d.** Mazuela; **e.** Tortozón; **f.** Zalema.

A similar variation is observed in cultivars Bobal, Hebén and Moscatel de grano menudo. Thus, Bobal and Hebén share with varieties in the Teta de vaca groups two characters: 1) The presence of seeds of a triangular shape, and 2) Two kinds of seeds by the shape of their apex. The first of these two aspects is also shared by Airén. Group Airén includes Airén, Bobal and Mazuela, and the relatively close values of aspect ratio make the two groups of Teta de Vaca and Airén closely related.

Cultivars with an acute beak: de Cuerno, Imperial, Macabeo, Mazuela, Tortozón, Zalema

The curvature analysis gave a constant pattern in these varieties whose beak is represented by a curve with maximum curvature in a single point (Fig. 7). In addition these varieties present higher maximum curvature values at their apex.

Cultivars with a plane or obtuse beak: Airén, Alarije, Albillo Real, Beba, Bruñal, Caíño Tinto, Castellana Blanca, Cayetana Blanca, Doña Blanca, Garnacha Tinta, Gewürztraminer, Graciano, Juan García, Listán Prieto, Malvasía Aromática, Mollar Cano, Monastrell, Moscatel de Alejandría, Palomino Fino, Pedro Ximénez, Prieto Picudo, Tortozona Tinta, Tempranillo

The images of the beaks in these cultivars end in a slightly convex, often almost flat upper line. The corresponding curves have in general two maximum values of curvature (Fig. 8). These cultivars belong to four morphological groups: Listán Prieto, Albillo Real, Moscatel and Doña Blanca, and their maximum curvature values are lower than those in the previous group.

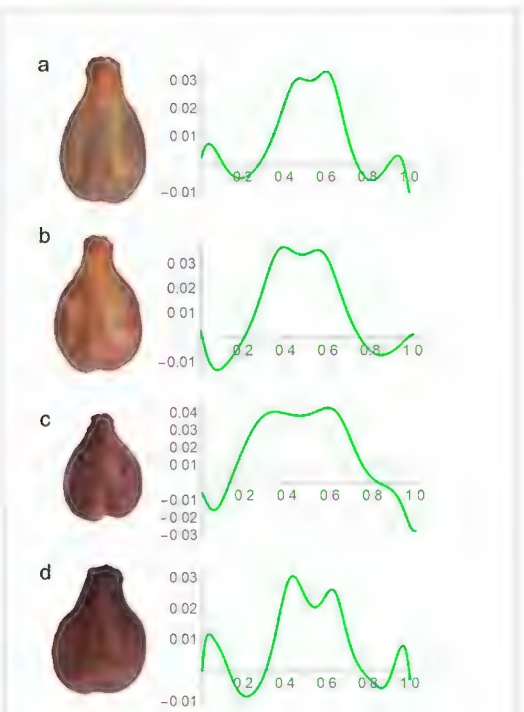

Figure 8. doi

Seed images and curvature values in the seed peduncle of cultivars: **a.** Doña Blanca; **b.** Mollar Cano; **c.** Tempranillo; **d.** Tortozona tinta.

Discussion

Recently, we proposed a classification of 38 cultivars of *Vitis vinifera* in 10 morphological groups, based on their similiarity with geometric models, obtained from the representation of equations, derived from models M7 and M8, described in previous work (Martín-Gómez et al. 2020b, Cervantes et al. 2021). The analysis described here complements these results and confirms the validity of the classification by morphological types grouped around geometric models. The work, here presented, departs from the representation of Bézier Curves corresponding to a set of points selected along the profile of the seed apex and the subsequent analysis of curvature values in these Curves (Cervantes and Tocino 2005, Noriega et al. 2008, Noriega et al. 2009, Tocino and Cervantes 2008). This method has been applied to seeds of the 38 cultivars used in the classification by morphological groups (Cervantes et al. 2021) and the results show the peduncles of *Vitis* seeds classified into three groups: 1) Acute, with a unique point of maximum curvature; 2) Plane, with two equivalent points of maximum curvature and 3) Intermediate, with a maximum point and a shoulder. Morphological groups, based on geometric models, are divided by the curvature analysis in series according to the type of curvature in their peduncles.

In the majority of the cultivars, the seeds had their pedicels flat at their apex. In consequence, representations of Bézier of their profiles had a plane form with two maximum curvature values. This type was observed in a total of 23 cultivars, including all but one of the 23 cultivars in four groups and with the addition of Airén. The cultivars with a flat pedicel are predominant in groups Listán Prieto (Listán Prieto and Tortozona Tinta), Albillo Real (Alarije, Albillo Real, Cayetana Blanca, Graciano, Juan García and Tempranillo), eleven of the twelve cultivars of group Moscatel (all of them except Moscatel de grano menudo), the three cultivars of group Doña Blanca (Doña Blanca, Monastrell and Pedro Ximénez) and Airén. Thus, morphological groups, Listán Prieto, Albillo Real, Moscatel and Doña Blanca, share in common the property of a peduncle plane at the top, whose Bézier representation presents two maximum curvature values. In consequence, these four groups constitute a series, ordered by decreasing values of Stummer Index from Listán Prieto to Doña Blanca. The remaining six groups of the morphological classification have varying proportions of plane and acute peduncles.

The seeds having their apex acute belong to six cultivars in four groups: Hebén (Macabeo and Zalema, but not Hebén itself), Tortozón (Imperial and Tortozón), Airén (Mazuela) and de Cuerno.

Nine cultivars remain to complete the total of 38 used in this work. These are the three *sylvestris* varieties (CA 13.4, CA 13.6 and SE 2.1), the three cultivars of group Teta de Vaca (Dominga, Teta de Vaca and Verdejo) and three cultivars that share with this group the peculiarity of having a mixture of seeds of the two types (Bobal, Hebén and Moscatel de grano menudo).

Wild type seeds have particular morphological aspects. They are of reduced size and with a small beak and, in consequence, their images have high values of roundness and Stummer Index with low Aspect Ratio (This et al. 2006, Viala and Vermorel 1901, Stummer

1911, Planchon 1887, Rivera et al. 2007). Thus, morphological analysis by the Elliptic Fourier Transform Method reveals wild type varieties clustering (Terral et al. 2009). Nevertheless, the separation is not absolute because some cultivars overlap with the *sylvestris* grapes (Terral et al. 2009).

Cultivars Dominga, Teta de vaca and Verdejo share a triangular shape and contain seeds of the two types: acute and plane at the top of the peduncle. The presence of the two morphological types is also a characteristic of Bobal, Hebén and Moscatel de grano menudo.

Curvature analysis allows us to discriminate amongst cultivars that gave similar results with the analysis based on geometric models. Thus, Macabeo and Zalema grouped with Hebén in the analysis of seed shape by comparison with geometric models. The curvature analysis in the beak gave a constant pattern consisting of a single curve with maximum curvature defined in a single point of maximum curvature values for Macabeo and Zalema, while there is a mixture of types (single and double maximum curvature values) in different seeds of Hebén.

The process of domestication of *V. vinifera* resulted in an increase in the size of berries and seeds accompanied by increased carbohydrate accumulation (Terral et al. 2009, This et al. 2006). In most cases, this has been parallel to increased cuticle deposition and thickening of the pedicel, broadening the top of the beak and resulting in this characteristic shape observed that results in two points of maximum curvature. Almost all cultivars of four groups had their seeds with the pedicel plane at the top (the two cultivars in Listán Prieto, six in Albillo Real, eleven out of twelve in Moscatel and all three in Doña Blanca). The only exception in all these four groups is Moscatel de Grano menudo and this can be explained because their seeds have long fragments of cuticle detached from the rib giving them the apparence of acuteness.

The morphological difference between the seeds of wild grapes and cultivars of *Vitis* has been known for long time, but biochemical and structural properties associated with these types remain to be investigated. Considering that lignin is an important component of the cell walls, it is possible that adaptation to agricultural conditions is associated with changes in lignin composition. In the pedicel, the xylem may be considered as mostly composed of protoxylem pre-veraison and later, on both proto- and metaxylem post-veraison (Chatelet et al. 2008a, Chatelet et al. 2008b). Pedicel thickening may be accompanied by increased number of secondary xylem cells and their lignification, similarly to what has been reported in the rachis (Nakamura and Hori 1985). Metabolic reactions in these processes will be differently regulated in the cultivars that have their beaks plane in comparison with those varieties that present acute beaks, resulting in an increased deposit of material in the former.

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